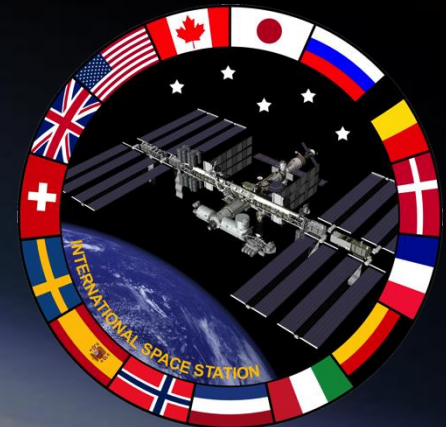
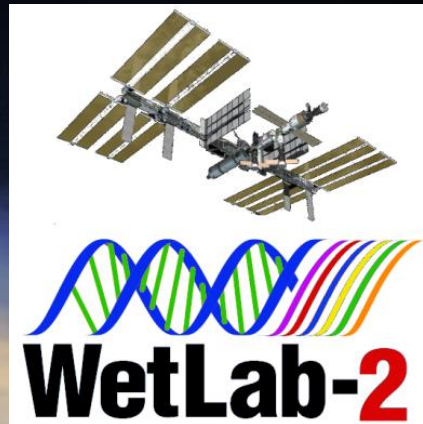




WetLab-2: Providing Quantitative PCR Capabilities on ISS



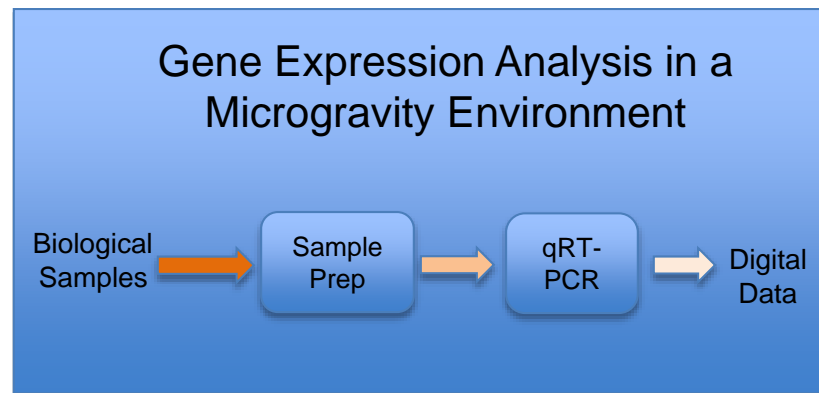
Macarena Parra
Ames Research Center
July 9, 2015



WetLab-2 Objectives

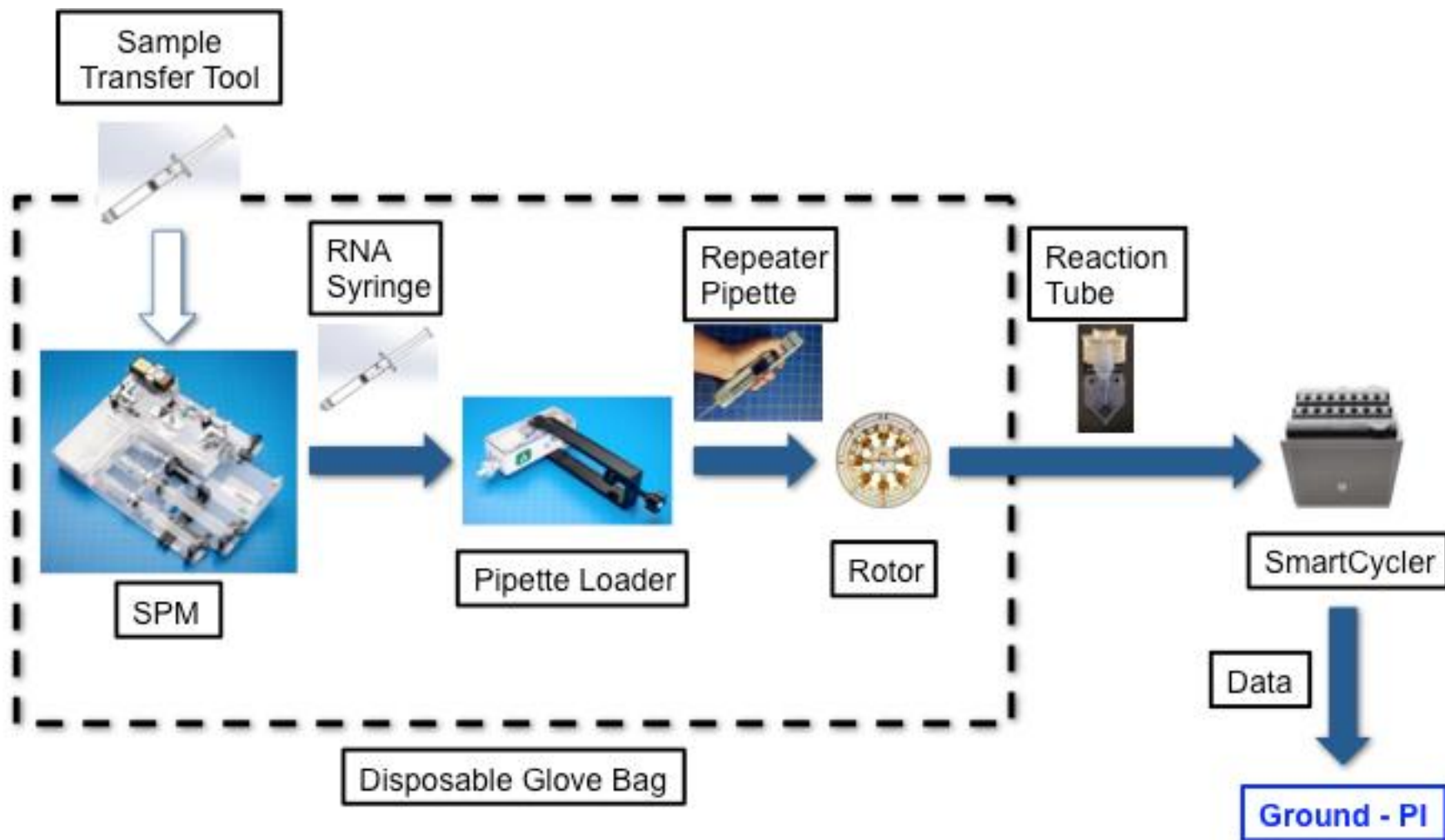


- To place on the ISS a research platform to facilitate space biology gene expression research.
 - Capability to process samples and perform qRT-PCR
- Facility will support multiple sample types (bacteria, cells, tissue)
- The analyzer will remain on ISS, while the experiment-specific disposable hardware will launch with the experiments.
- Also capable of supporting analysis of air, surface, water, and crew health.
- Validation Flight: SpaceX-7





WetLab-2 Operations Overview





Sample Transfer Tool



- WL2 uses a Luer-lok connection to accept samples
- Techshot Analytical Containment Transfer Tool (ACT2)
 - Uses luer-lok
 - Provides two levels of containment
 - Maintained throughout sample transfer process
- Standard syringe can be used if two containment levels are not needed

For Validation Flight:

- Will use the 5ml configuration of the ACT2
- Sample (*E. coli*) will be frozen at -80C after loading in unit
- Crew will thaw the sample then use it as input to the SPM
- Will use a standard luer-lok syringe to introduce the tissue

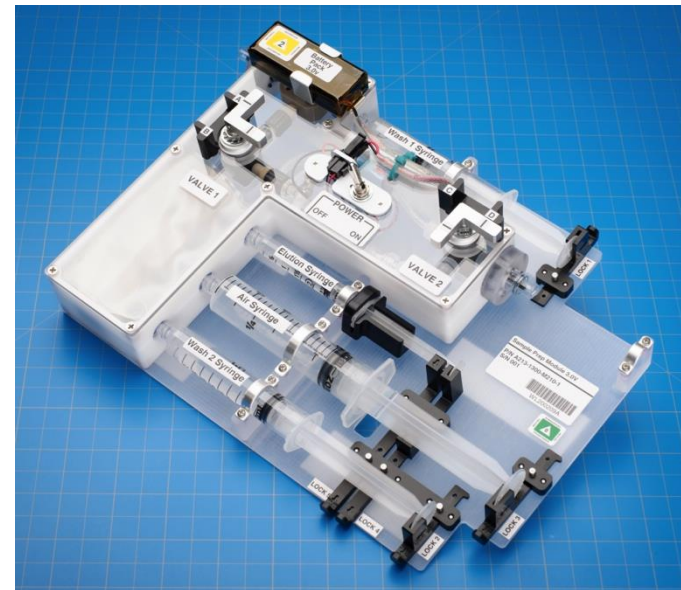




Sample Preparation Module (SPM)



- Function of SPM
 - Breaks open cells and binds RNA to column
 - Washes RNA
 - Elutes RNA into removable RNA syringe
- Two versions: mammalian, bacterial
- Closed system
- Fluids are pre-loaded in syringes
- No alcohols or organic solvents
- Disposable one time use
- Designed to be run in Disposable Glove Bag (DGB)
- Crew manipulations consist of:
 - Attaching and removing syringes
 - Turning OmniLyser on and off
 - Pushing syringe plungers
 - Switching valves



**System has been
successfully tested on the
ground with bacterial and
mammalian cells**

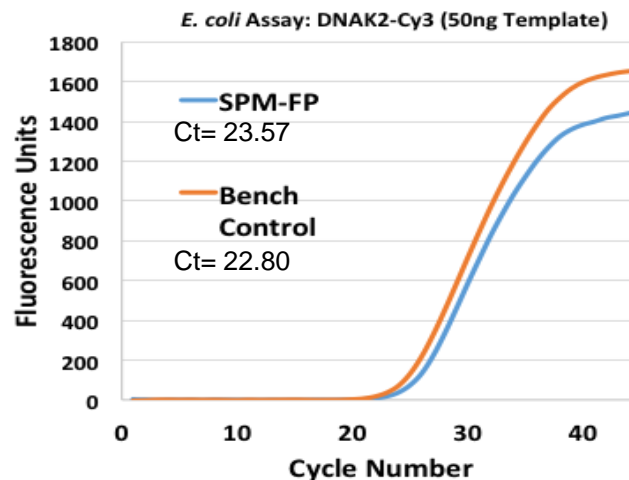
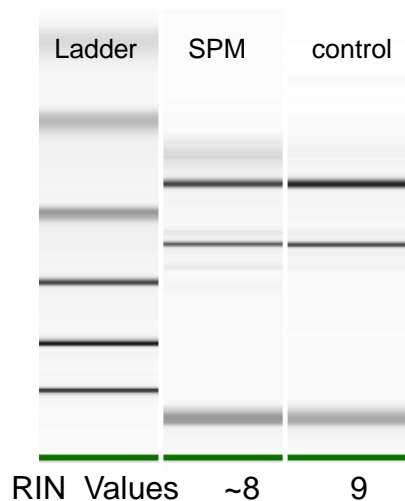


Sample Preparation Module (SPM)

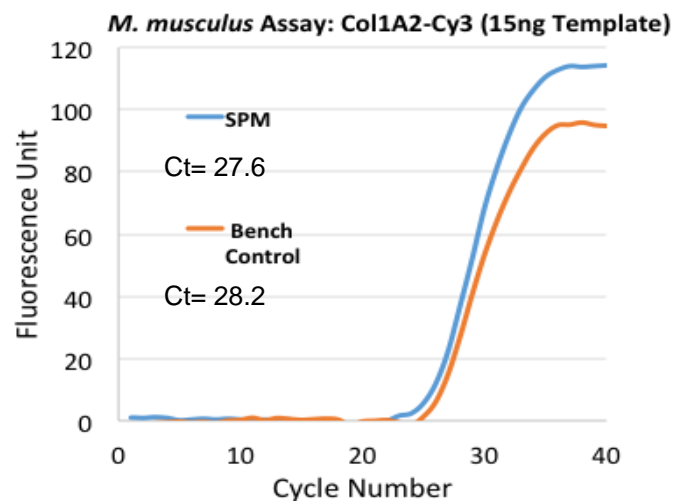
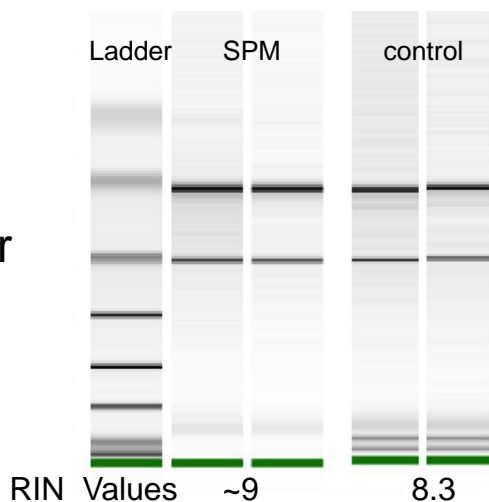


SPM runs give comparable results to those from the already proven ClaremontBio bench procedure

E. coli



Mouse liver

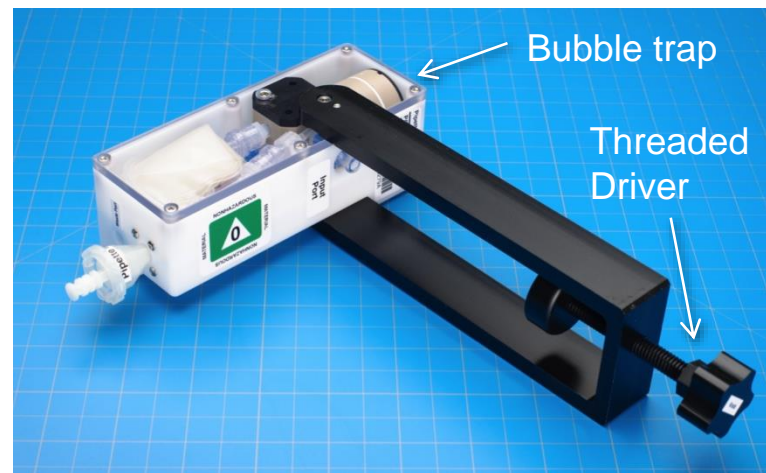




Pipette Loader



- Function of RAM (Pipette Loader)
 - Removes air bubbles from the RNA sample
 - Loads pipette tip
- Closed system
- Designed to be run in the Disposable Glove Bag (DGB)
- No fluids pre-loaded (long shelf life)
- Sample must be driven slowly through the bubble trap to be effective
- Loads the sample into the Repeater Pipette Tip for downstream tube loading





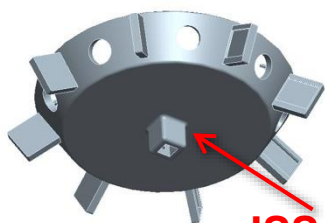
Reaction Tube Loading and Rotor



- Reaction Tube Loading
 - Pipette is used to load 25ul into each Reaction Tube
 - Loading occurs through septum of modified cap
 - Prototypes tested on Parabolic Flight
- Reaction Tube Rotor
 - Holds 8 Reaction Tubes
 - Rotor is attached to drill on ISS
 - Spin to get RNA into the sample window
 - Prototypes used on Parabolic Flight – design changes made based on experience



Sample Window



ISS Drill Interface





Pre-filled Reaction Tubes



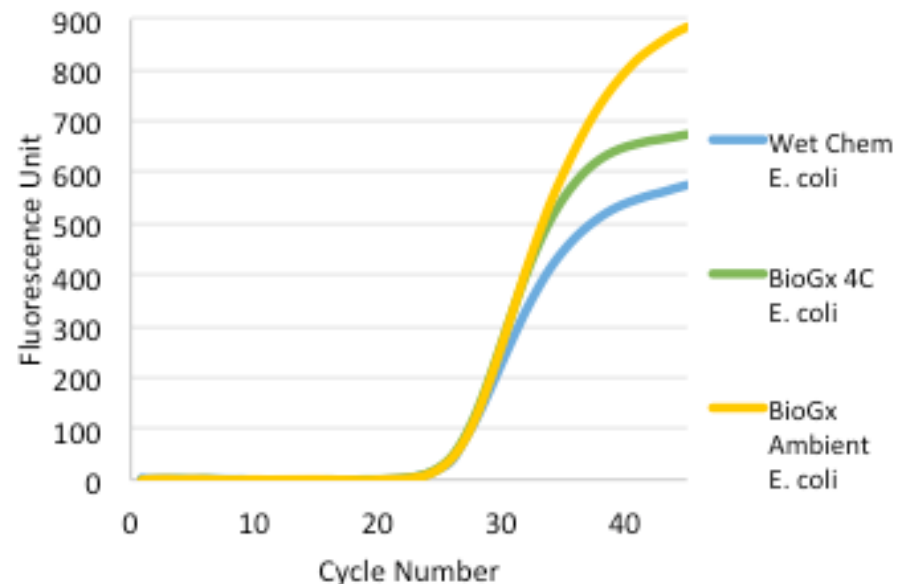
- Pre-filled Reaction Tubes
 - Tubes are a COTS SmartTube with a custom septa cap for loading in microgravity
 - Tubes contain lyophilized primers, probes, enzymes and Master Mix
 - Tubes will be foil packed to protect from moisture and light
 - Lyophilized Reagents give comparable data to commercial wet chemistry reagents



Lyophilized qPCR Assay



NASA Formulated BioGx Lyophilized Reagents





SmartCycler Hardware

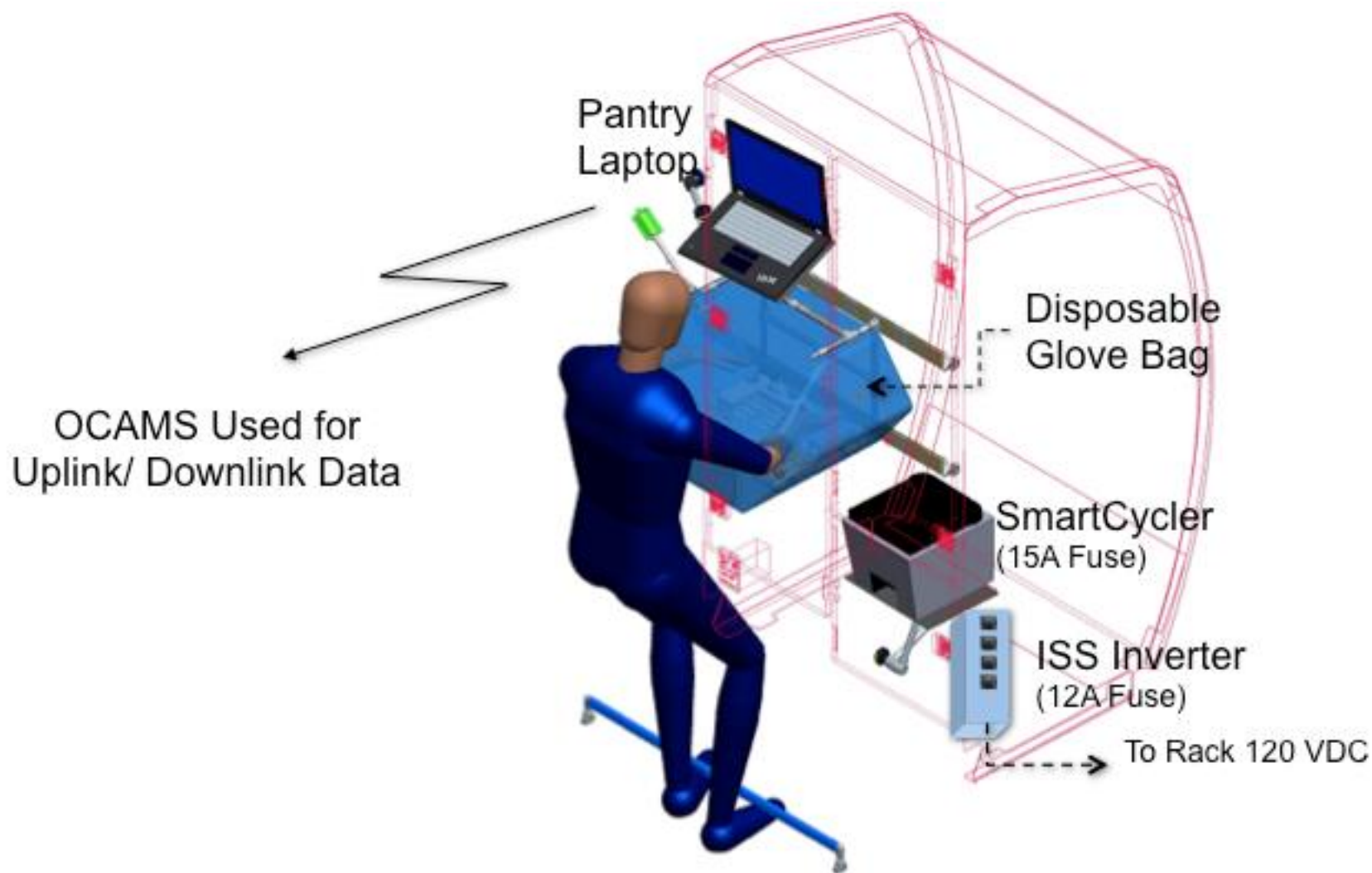


- SmartCycler
 - qRT-PCR system
 - Instrument is designed for field work
 - Will fly as a COTS item
 - Will be used in the aisle
 - Mounted on a Bogun Arm on the rack
 - Provides 16 wells and multiplexing capability
 - Thermal programs can be uploaded from the ground
 - Data can be downlinked to ground after run





On-Orbit Configuration





Validation Flight – SpaceX-7



Goal of Validation Flight: On-orbit test and check-out of the WetLab-2 system in a systematic way to ensure it will return valid data to future researchers

Objectives of Validation Flight:

- Install software and set-up hardware (Session 1)
- Does real-time PCR data generated on-orbit match data on earth? (Session 2)
 - No convection or other microgravity related issues
 - Validate SmartCycler, RAM, tube loading and rotor functions
- Does the Sample Processing Module function correctly on-orbit? (Session 3)
 - All fluidic manipulations function properly
 - Prove out system with first sample type (*E. coli*)
 - Test system using on-orbit isolated RNA as input to SmartCycler
- Does system function correctly on-orbit with tissues? (Session 4)
 - All fluidic manipulations function properly
 - Prove out system with second sample type: mouse tissue

Flight results from each session will be compared to results from ground controls
Ground controls will be run with a 2-24 hour delay from the flight samples



End to End Test Data



Session 3: *E. coli* qRT-PCR

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Cy3 Ct	Cy5 Ct	Avg A	SD	Avg B	SD	Avg C	SD
A1	Session 3	Gene A <i>singleplex</i>	E. coli RNA	19.87	0	0	20.08	0.25				
A2	Session 3		E. coli RNA	20.06	0	0						
A3	Session 3		E. coli RNA	20.44	44.44	0						
A4	Session 3		E. coli RNA	19.94	35.29	0						
A5	Session 3	Gene B <i>singleplex</i>	E. coli RNA	0	20.44	0			20.29	0.41		
A6	Session 3		E. coli RNA	0	20.13	0						
A7	Session 3		E. coli RNA	0	20.78	0						
A8	Session 3		E. coli RNA	0	19.82	0						
A9	Session 3	Genes A&B <i>duplex</i>	E. coli RNA	20.03	20.35	0	19.75	0.42	20.40	0.15		
A10	Session 3		E. coli RNA	19.32	20.58	0						
A11	Session 3		E. coli RNA	19.47	20.22	0						
A12	Session 3		E. coli RNA	20.19	20.43	0						
A13	Session 3	Genes A, B, & C <i>triplex</i>	E. coli RNA	20.24	20.75	22.55	19.96	0.89	20.97	0.69	22.98	1.02
A14	Session 3		E. coli RNA	20.09	21.71	23.29						
A15	Session 3		E. coli RNA	20.81	21.3	24.24						
A16	Session 3		E. coli RNA	18.71	20.12	21.85						

Successful singleplex, duplex and triplex results

This data is typical for post-optimization runs



End to End Test Data



Session 4: mouse liver qRT-PCR

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Cy3 Ct	Cy5 Ct	Avg A	SD	Avg B	SD	Avg C	SD
A1	Session 4	Gene A	mouse liver RNA	22.68	0	0	22.29	0.35				
A2	Session 4	<i>singleplex</i>	mouse liver RNA	21.83	0	0						
A3	Session 4		mouse liver RNA	22.3	0	0						
A4	Session 4		mouse liver RNA	22.36	0	0						
A5	Session 4	Gene B	mouse liver RNA	0	22.15	0			21.62	0.44		
A6	Session 4	<i>singleplex</i>	mouse liver RNA	0	21.07	0						
A7	Session 4		mouse liver RNA	0	21.66	0						
A8	Session 4		mouse liver RNA	0	21.61	0						
A9	Session 4	Genes A&B	mouse liver RNA	23.47	21.61	0	23.86	0.72	21.55	0.15		
A10	Session 4	<i>duplex</i>	mouse liver RNA	23.79	21.48	0						
A11	Session 4		mouse liver RNA	24.89	21.72	0						
A12	Session 4		mouse liver RNA	23.27	21.37	0						
A13	Session 4	Genes A, B, & C	mouse liver RNA	26.51	23.32	25.83	26.58	0.85	23.38	0.38	26.41	1.23
A14	Session 4	<i>triplex</i>	mouse liver RNA	25.64	23.2	25.71						
A15	Session 4		mouse liver RNA	27.7	23.06	25.84						
A16	Session 4		mouse liver RNA	26.48	23.93	28.26						

Successful singleplex, duplex and triplex results

This data is typical for post-optimization runs



Conclusion



- WetLab-2 will facilitate space biology gene expression research
 - Establishes a qRT-PCR analytical instrument on the ISS.
 - Provides a Standard Transfer System for sampling among Wetlab-2 and other bioprocessing & analytical systems (Techshot ACT2)
 - Sample preparation of minimal complexity, can be completed by crew in <2 hours
- Reduce need for downmass of samples due to on-orbit analysis
- Allow researchers to begin to utilize the ISS as a fully working laboratory
 - Results will be available to researchers within hours of run completion allowing for the potential for interactivity with experiments driven by the analysis of results
- Provide on-orbit analysis of air, surface, water, and clinical samples to monitor environmental contaminants and crew health.
 - Results would be available in as little as 90 min compared to current testing that takes 3-6 months due to the need for sample return
- Looking for users of the system after completion of validation flight



WetLab-2 Team



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Gary Hiatt

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Ops POC:

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RPM:

Jessica Curry

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Matt Chin
Matt Everingham

Test Leads

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Jennifer Murphy

Operations

Jessica Hauss
Cindy Harris

Finance

Veny Jubilo

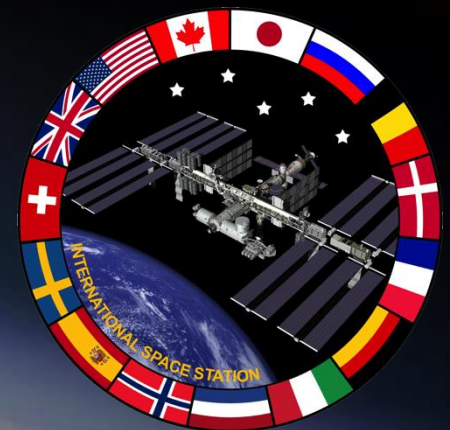
Manufacturing

Emmett Quigley
Ron Strong

Note: Wetlab-2 = Wet Lab RNA SmartCycler



Backup Slides





End to End Test Data

Session 2: q-PCR using QC DNA

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Avg	SD
A1	Session 2	Low Template	E.coli DNA	30.25	29.72	0.38
A2	Session 2		E.coli DNA	29.71		
A3	Session 2		E.coli DNA	29.35		
A4	Session 2		E.coli DNA	29.57		
A5	Session 2	Mid Template	E.coli DNA	22.6	22.55	0.15
A6	Session 2		E.coli DNA	22.59		
A7	Session 2		E.coli DNA	22.67		
A8	Session 2		E.coli DNA	22.33		
A9	Session 2	High Template	E.coli DNA	16.04	16.30	0.45
A10	Session 2		E.coli DNA	16.21		
A11	Session 2		E.coli DNA	16.95		
A12	Session 2		E.coli DNA	15.99		
A13	Session 2	No Template	E.coli DNA	0	0	0
A14	Session 2		E.coli DNA	0		
A15	Session 2		E.coli DNA	0		
A16	Session 2		E.coli DNA	0		

No carryover from tube to tube

This data is typical



Post-Validation/Post-Flight



- Fluidics components are disposable
- SmartCycler to remain on board ISS
- SLPS Principle Investigators who propose to use the SmartCycler with launch fluidics components with science hardware
 - Current estimates indicate SmartCycler use 2-4 investigations per year
 - Reagents loaded in SPM and SmartTubes to be experiment-specific